1: Eur J Biochem. 1989 Jun 15;182(2):349-56.

Nitrilase of Rhodococcus rhodochrous J1. Purification and characterization.

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Nitrilase was purified from an extract of isovaleronitrile-induced cells of Rhodococcus rhodochrous J1 in seven steps. In the last step, the enzyme was crystallized by adding ammonium sulfate. The crystallized enzyme appeared to be homogeneous by polyacrylamide electrophoresis, ampholyte electrofocusing and double immunodiffusion in agarose. The enzyme has a molecular mass of about 78 kDa and consists of two subunits identical in molecular mass. The purified enzyme exhibits a pH optimum of 7.6 and a temperature optimum of 45 degrees C. The enzyme catalyzed stoichiometrically the hydrolysis of benzonitrile to benzoic acid and ammonia, and no formation of amide was detected. The enzyme required thiol compounds such as dithiothreitol, L-cysteine or reduced glutathione to exhibit maximum activity. The enzyme was specific for nitrile groups attached to an aromatic or heteroaromatic ring, e.g. benzonitrile, 3-chlorobenzonitrile, 4-tolunitrile, 2-furonitrile and 2-thiophenecarbonitrile. The comparison of the properties of the enzyme with other nitrilases and nitrile hydratases has been also discussed.

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